Evaluation of ULV Applications Against Old World Sand Fly (Diptera: Psychodidae) Species in Equatorial Kenya

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ABSTRACT Reducing populations of phlebotomine sand flies in areas prevalent for human leishmaniases is of ongoing importance to United States military operations and civilian populations in endemic regions. However, not enough is known regarding the efficacy of Department of Defense-approved pesticides and equipment against sand flies; specifically, the potential for ultra-low volume (ULV) pesticide applications to control Old World sand fly vectors. In this study we examine two sprayers, the Terminator ULV and the Grizzly ULV, with UV-labeled Duet and Fyfanon in four combinations against caged *Phlebotomus duboscqi* (Neveu-Lemaire) and wild sand fly populations in a natural environment in western Kenya. All equipment and Fyfanon have United States military National Stock Numbers and both pesticides are registered with the United States Environmental Protection Agency. Caged sand flies were reared from local *P. duboscqi* and the area has long been studied because of high incidences of human cutaneous and visceral *Leishmania*. Patterns of mortality across grids of caged sand flies showed greater efficacy from the Grizzly ULV regardless of chemical. The Terminator ULV performed well with Duet but with a less uniform and overall lower rate of mortality across the spray grid. Sampling of wild populations before and after treatments suggested local population suppression from ULV treatments, as well as a possible repellent effect in nearby untreated areas. Surprisingly, ULV active ingredient deposition inferred from patterns of UV-labeled droplets captured on cotton ribbons adjacent to sand fly cages in spray plots did not match patterns of mortality. We discuss the implications of this study, the first of its kind, for future military preventive medicine activities, including relative performance costs and benefits of larger or smaller sprayers, and the relative stability of ULV-induced mortality patterns in varied or sub-optimal conditions.

KEY WORDS Marigat, Kenya; phlebotomine sand fly; Fyfanon; Duet; Deployed War Fighter Protection Program

Sand fly vectors of human *Leishmania* parasites, in particular sand flies in the genus *Phlebotomus,* pose a substantial threat to the health of U.S. troops deployed to arid regions in the Middle East and Africa, and to civilian populations in endemic regions of the world. Reducing populations of phlebotomine sand flies in areas prevalent for *Leishmania* is of ongoing importance to U.S. military operations. Historical accounts of collateral reduction of sand flies, human cases of leishmaniasis, or both during massive pesticide campaigns against vectors of malaria indicate that residuals such as DDT can be effective (Nadim and Amini 1970). Large scale programs of applying residual pesticides other than DDT targeted against sand flies are still used by many countries and international agencies, and by the U.S. military in Iraq and Afghanistan (Coleman et al. 2006), but these programs can be difficult to sustain (Alexander and Maroli 2003) and should be only one element in a multifaceted system to reduce sand fly populations and human cases of visceral and cutaneous leishmaniasis (Britch et al. 2009, 2010a,b). Residual pesticide applications should be used in conjunction with aerosol applications such as ultra-low volume (ULV) pesticide dispersal (Lofgren 1970), habitat reduction such as removal of rodent burrows (Faizulin et al. 1976), physical exclusion such as bed nets (Zollner et al. 2007), and personal protective measures such as application of DEET to exposed skin and permethrin treatment of clothing (Coleman et al. 2006; reviewed in Kitchen et al. 2009) to form an integrated vector management system of defense against sand flies. However, from the perspective of U.S. military pest man-

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14. ABSTRACT

Reducing populations of phlebotomine sand ?ies in areas prevalent for human leishmaniases is of ongoing importance to United States military operations and civilian populations in endemic regions. However, not enough is known regarding the ef?cacy of Department of Defenseapproved pesticides and equipment against sand ?ies; speci?cally, the potential for ultra-low volume (ULV) pesticide applications to control Old World sand ?y vectors. In this study we examine two sprayers, the Terminator ULV and the Grizzly ULV, with UV-labeled Duet and Fyfanon in four combinations against caged Phlebotomus duboscqi (Neveu-Lemaire) and wild sand ?y populations in a natural environment in western Kenya. All equipment and Fyfanon have United States military National Stock Numbers and both pesticides are registered with the United States Environmental Protection Agency. Caged sand ?ies were reared from local P. duboscqi and the area has long been studied because of high incidences of human cutaneous and visceral Leishmania. Patterns of mortality across grids of caged sand ?ies showed greater ef?cacy from the Grizzly ULV regardless of chemical. The Terminator ULV performed well with Duet but with a less uniform and overall lower rate of mortality across the spray grid. Sampling of wild populations before and after treatments suggested local population suppression from ULV treatments, as well as a possible repellent effect in nearby untreated areas. Surprisingly, ULV active ingredient deposition inferred from patterns of UV-labeled droplets captured on cotton ribbons adjacent to sand ?y cages in spray plots did not match patterns of mortality.Wediscuss the implications of this study, the ?rst of its kind, for future military preventive medicine activities, including relative performance costs and bene?ts of larger or smaller sprayers, and the relative stability of ULV-induced mortality patterns in varied or sub-optimal conditions.

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agement systems, not enough is known regarding the efficacy of the current arsenal of Department of Defense (DoD)-approved pesticides and equipment, as well as other U.S. Environmental Protection Agency approved pesticides that may not yet be in the DoD National Stock Number system, against phlebotomine sand flies (Linthicum et al. 2007, Cope et al. 2008, Dalton 2008, Kitchen et al. 2009). In particular, we need to know more about the potential for ULV-delivered pesticides to control Old World sand fly vectors of *Leishmania.*

Ultra-low volume aerosol dispersal of insecticides against insect disease vectors has been carried out for over 60 yr and is used routinely in mosquito control operations worldwide (Lofgren 1970, Mount 1998). Although the efficacy of ULV against mosquito vectors has been well studied in many environments (reviewed in Britch et al. 2010a), no studies have been identified that rigorously examine the efficacy of ULV insecticide application against sand flies. Insecticidal treatments using malathion or resmethrin with a truck mounted ULV sprayer, or permethrin with a hand held ULV sprayer were used as part of a comprehensive and well documented sand fly eradication program at Tallil Air Base during Operation Iraqi Freedom (Coleman et al. 2006). However, there was little evidence found of significant reductions in sand flies captured in outdoor traps after extensive and repeated area spray treatments, although longevity of individual adult sand flies may have been affected with a commensurate decrease in vectorial capacity for *Leishmania* transmission. Explanations for the lack of significant reduction in sand fly populations from ULV applications at Tallil Air Base include replenishment of sand fly populations from outlying untreated areas, timing of ULV sprays not coinciding with adult sand fly activity, or the possibility that sand flies were not susceptible to the pesticides or the method of delivery of the pesticides (Coleman et al. 2006).

In the current study we look at the efficacy of ULV applications against *Phlebotomus duboscqi* (Neveu-Lemaire), an Old World sand fly vector of *Leishmania,* in a natural environment in equatorial western Kenya. We compare two ULV generators and two ULV-formulated pesticides in four combinations against sentinel colony-reared sand flies as well as local wild sand fly populations. Both ULV generators and one of the pesticides are approved and listed by the DoD Armed Forces Pest Management Board (AFPMB 2011), and both pesticides are registered by the U.S. Environmental Protection Agency for the control of mosquitoes and other biting flies.

Materials and Methods

Study Site. The study site was a field at the Kenya Agricultural Research Institute (KARI) Marigat Field Station located in a hot semiarid agricultural area ≈1.5 km east of the town of Marigat and ≈12 km south west of Lake Baringo, with a mean annual rainfall of ≈654 mm and mean high temperature of ≈32°C (KARI 2011). Marigat is at just over 1,000-m elevation and ≈50 km north of the equator in western Kenya at the heart of the Rift Valley. This area has long been studied as a focus of *Leishmania* (McKinnon and Fendall 1955, Mutinga and Ngoka 1983, Lawyer et al. 1990) and *Leishmania* vectors (Minter 1964), with abundant wild populations of *Phlebotomus* species present, in particular *P. martini* Parrot and *P. duboscqi* (Kasili et al. 2010). We used the longest section of an L-shaped field (centered at 0.470347° N, 36.000474° E) running nearly 270 m long east to west and nearly 65 m wide north to south, bordered by a thin margin of forest and surrounded by similar agricultural fields, dirt roads, and small irrigation canals (Fig. 1A). The ground is level with old, packed down furrows running east-west with a central 0.2-m-tall and 2.0-m-wide mound running the entire length east to west, and vegetation consisted of grasses <0.1 m tall and a few scattered forbs <0.25 m tall.

Sprayers and Spray Materials. We used two ULV generators in this study, the Clarke Grizzly Cold Aerosol Generator (Clarke Mosquito Control Products, Roselle, IL) and the Terminator Diesel ULV Sprayer/Compressor (ADAPCO Solutions & Technology, Sanford, FL). The Grizzly is a 207-kg truck-mounted ULV system powered by a 13.4 kW Briggs & Stratton gasoline engine that drives a rotary positive displacement blower. This blower provides airflow of up to 10 m^3/min. The Grizzly has a laminar air flow (IHPLAT) nozzle and the sprayer's maximum flow rate is 500 ml/min. Laboratory evaluation by Hoffmann et al. (2007) found that the Grizzly produces $D_{V0.1}$ of 3.5 μm, $D_{V0.5}$ of 17.5 μm, and $D_{V0.9}$ of 38.6 μm, with 56.4% of droplets <20 μm when applying a test material of BVA 13 ULV oil. In the field we carried out a calibration run with the Grizzly on flow rate setting #7 and determined it to produce a flow rate of 303 ml/min for this study. The Terminator is a 65 kg truck- or all terrain vehicle (ATV)-mounted ULV system powered by a 3.5 kW Yanmar diesel engine that powers a direct-drive air compressor that produces the air blast through two venturi-style stainless steel nozzles. The compressor provides airflow at the rate of 0.3 m^3/min at 690 kPa, with a spray flow rate of 88.7–117.4 ml/min. Laboratory evaluation by Hoffmann et al. (2007) found that the Terminator with a No. 24 orifice produces $D_{V0.1}$ of 3.6 μm, $D_{V0.5}$ of 17.2 μm, and $D_{V00.9}$ of 38.8 μm, with 56.6% of droplets <20 μm when applying a test material of BVA 13 ULV oil. In the field we carried out a calibration run with the Terminator on the fixed flow rate setting and determined it to produce a flow rate of 60 ml/min for this study.

For spray materials, we used Fyfanon ULV (Cheminova, Inc., Wayne, NJ) and Duet dual action adulticide (Clarke Mosquito Control Products, Roselle, IL). Fyfanon is a 96.5% malathion oil-based formulation rated for ULV or thermal fog insecticide application. Malathion is one of the most frequently used organophosphate insecticides worldwide, targeted against a broad range of pest and disease vector insects. Duet is an oil soluble synergized synthetic pyrethroid adulticide containing prallethrin, Sumithrin, and technical piperonyl butoxide (PBO). The combination of prallethrin and Sumithrin in Duet is designed to cause agitation of mosquitoes after chemoreception of prallethrin, drawing them from a resting state to flying and thus greater contact with the aerosolized formulation (Cooperband et al. 2010). Both Fyfanon and Duet were used undiluted

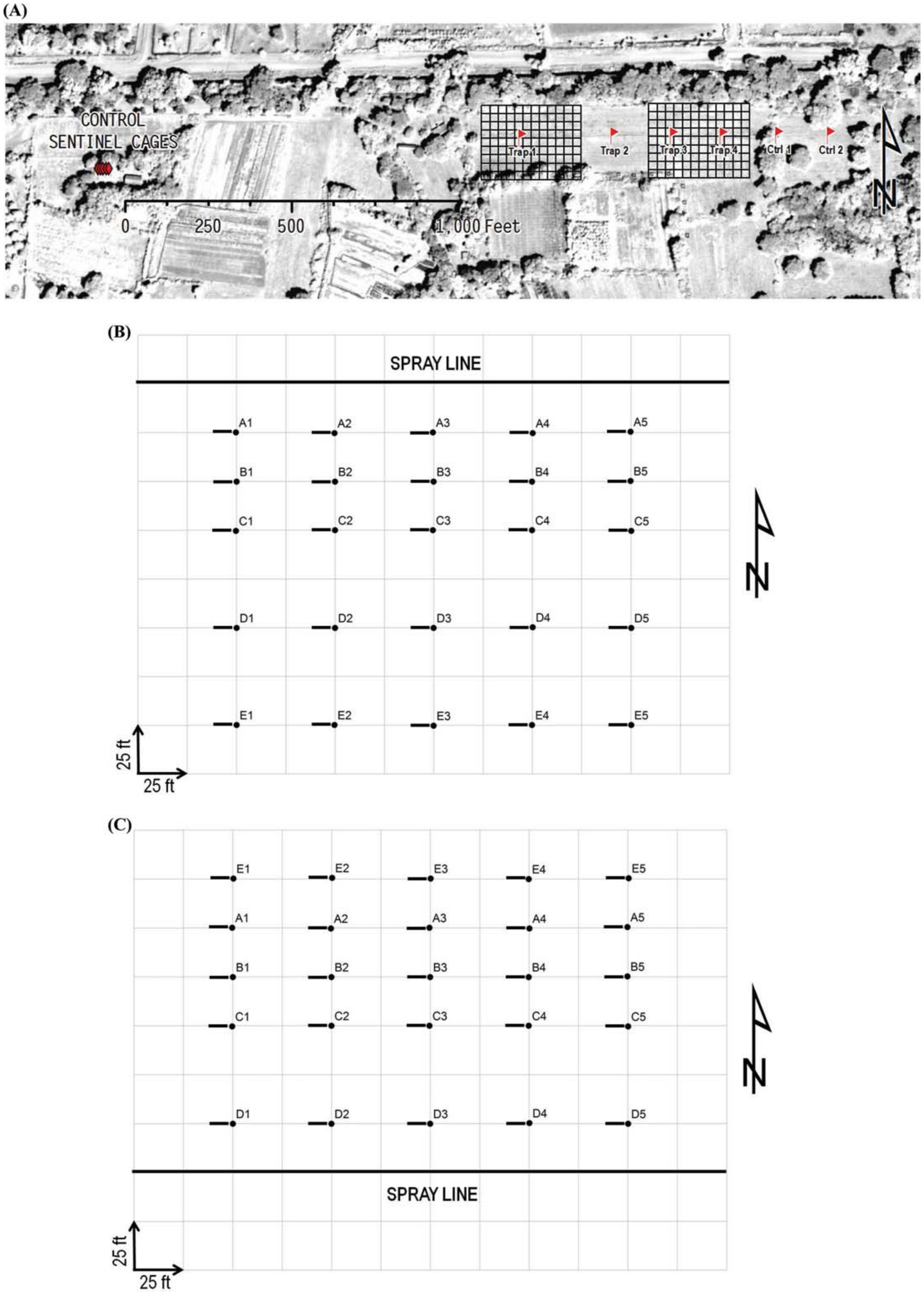


Fig. 1. (A) Area map showing control cage location (red diamonds) at the KEMRI/USAMRU-K field station, CDC traps within and between spray grids (red flags, Trap 1 – Trap 4), and CDC traps in untreated offsite areas to the east (red flags, Ctrl 1 and Ctrl 2). The spray areas are delineated with grids of 25 feet squares; the left hand grid is the west plot and the right hand grid is the east plot. (B) Cartogram of cage positions and ribbons centered 200 feet along and 175 feet downwind of 300 feet east-west spray line on spray day 1. (C) Cartogram of cage positions and ribbons, centered 200 feet along and 150 feet downwind of 300 feet east-west spray line on spray day 2.

in both ULV generators. We added Uvitex OB (Ciba Corporation, Newport, DE) UV-fluorescent dye at the rate of 4 g/liter to each tank of spray material to allow us to visualize droplets on cotton ribbon capture apparatus, described below, and to later use fluorometric quantitation to estimate droplet deposition across the spray field. Immediately before each spray run we removed 20 ml of dye/pesticide solution from the spray tank and applied it to 30 feet of cotton ribbon coiled in a plastic bag until it was completely absorbed, and allowed to dry. These ribbons were later fluorometrically analyzed to determine the exact concentration of dye in each spray run. This posthoc analysis was necessary because the Uvitex dye powder has been observed to show a range of solubility depending on spray material and concentration.

Test Grids of Sentinel Sand Fly Cages. To measure the efficacy of ULV applications we set up two grids of caged sentinel sand flies in the study area based on the experimental design first described in Rogers et al. (1957) and later modified as in Britch et al. (2010a). We deployed grids of 25 sentinel cages in a 150 feet (day 1) or 125 feet (day 2) by 200 feet array positioned 25 feet downwind of a 300 feet spray line (Fig. 1B and C). We used 122-cm white polyethylene Tread-In Posts (Jeffers, Dothan, AL) to support sentinel sand fly cages at the positions illustrated in Fig. 1. We affixed one cage to each post 61 cm above the ground by using a disposable hook and loop cable tie (Fig. 2A). In both plots, each post was labeled with a unique alphanumeric code from A1 to E5 (Fig. 1), and this code along with the date and type of application was copied on to each sentinel cage. In addition to the two grids of 25 sentinel test cages, we set up a line of five sentinel control cages at the Kenya Medical Research Institute/U.S. Army Medical Research Unit-Kenya (KEMRI/USAMRU-K) field station within the KARI property located >0.3 km to the west of the west plot (Fig. 1A).

The sentinel cages were made from cylindrical paper food containers (8.5 cm in diameter by 4.5 cm in depth; Neptune Paper Products, Newark, NJ) with the paper disk top and bottom replaced with amber Lumite screening (32 by 32 mesh per inch; Bioquip Products, Rancho Dominguez, CA) held in place with two rubber bands and the original paper lid bezel. The hook and loop cable tie is slipped through one of the rubber bands to hold the cage on the post (Fig. 2A, inset). Each sentinel treatment and control cage contained ≈20 colony reared *P. duboscqi* sand flies in a mixture of males and females. The sand flies used in the treatment and control sentinel cages were from a *P. duboscqi* colony reared in Marigat at the KEMRI/USAMRU-K field station from parent progeny that were 100% field caught in the Marigat area in 2007. The colony is periodically infused with new wild caught flies approximately once per year to counteract declines in colony numbers from mite activity during the cool seasons, the most recent infusion having been September, 2009. The colony is reared according to the KEMRI protocol using hamsters as a blood source. Although incubators called for by the WRAIR sand fly rearing protocol are not available at the Marigat research site, a room temperature of ≈28–30°C is maintained year round, using space heaters in the cool seasons when necessary.

We stored sentinel cages containing sand flies in coolers containing moistened towels during travel to the study area from the field station, and waited until ≈30 min before the anticipated spray time to affix cages to posts. After a wait time of 10 min after the end of each spray sentinel cages were removed from the posts and transported in coolers directly to the KEMRI/USAMRU-K field station where the 10-min mortality for each sentinel cage was immediately recorded. Surviving sand flies were transferred to new cages and supplied with a sugar water source. We recorded subsequent mortality at 12 h and 24 h postspray for each cage. Control cages were handled separately but with timing, storage, and mortality tallying methods identical to cages used in the spray plots. We calculated the mean, range, median, and interquartile range (IQR) for all mortality data by plot and spray trial with Microsoft Excel. The IQR is given in data units and is a measure of the spread of data around the median value, bound by the 25th and 75th percentiles. Thus, the IQR ignores outliers above and below the most frequently observed values. Low IQR values indicate that the majority of observations fall into a narrow range around the median value because a treatment generally caused a consistent response. However, high IQR values indicate that data are more broadly scattered owing to a noticeable variability in the outcome of a treatment.

Standard CDC downdraft incandescent light traps (John W. Hock Co., Gainesville, FL) baited with CO_2 from dry ice were positioned at six locations running west to east through the study area (Fig. 1A). We placed one trap in the west plot, one trap between the plots, and two traps in the east plot. We also placed two additional traps further to the east in an untreated area. We ran these traps for two nights preceding the first spray, the night immediately after the first spray, the night immediately after the second spray, and finally one night thereafter to obtain a relative measure of efficacy of the ULV applications on local wild sand fly populations dominated by *P. duboscqi*, *P. martini*, and *Sergentomyia schwetzi* Adler, Theodor, & Parrot. We predicted that wild sand fly numbers would drop postspray in both treated plots, but would remain stable postspray at locations sampled to the east in untreated areas.

Both the Fyfanon and the Duet were used in each ULV generator, totaling four separate runs on the two experimental plots of caged sentinel sand flies. On the first spray day, the Terminator was run with Duet at the label rate of 1.23 oz (undiluted) per acre, from west to east on the north edge of the west spray grid, and the Grizzly was run with Fyfanon at a rate of 3.5 oz (undiluted) per acre, from east to west on the north edge of the east spray grid (Figs. 1B and 3A). With the wind primarily from the north-northeast at 1.4–2.1 mph, the opposing travel directions allowed us to run the sprays simultaneously in the relatively small available study area with less concern of cross-contamination of plots. On the second spray day, the Terminator was run with Fyfanon at 3.6 oz/acre, from east to west on the south edge of the

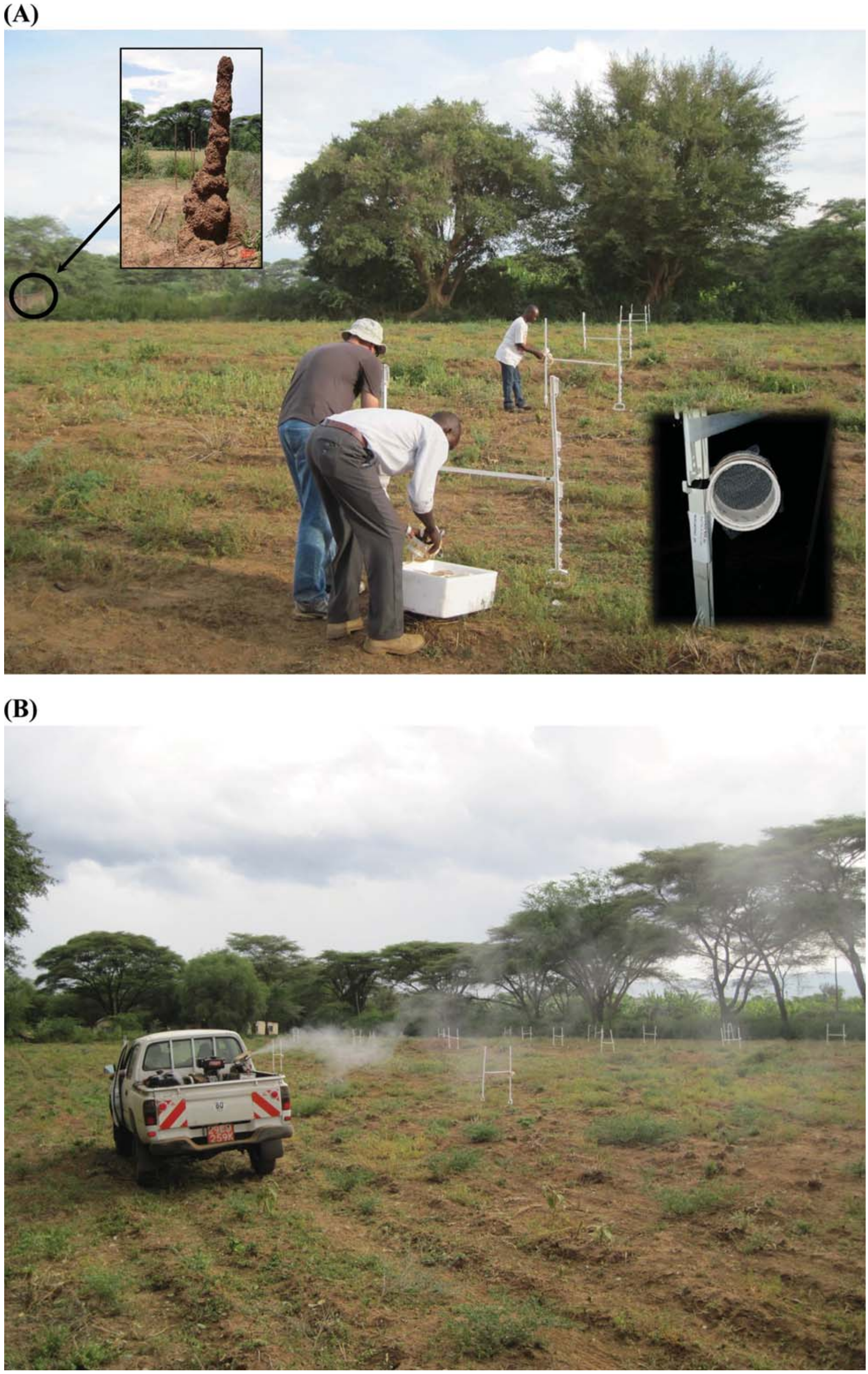


Fig. 2. (A) Sentinel sand fly cages being removed from coolers and affixed to posts in the west plot on the second spray day; looking south. Cotton droplet capture ribbons have already been attached in position between the posts at each cage position. Upper left inset shows approximate location of one of the termite mounds (2.5 m high); lower right inset shows close-up of sentinel cage and adjacent cotton ribbon attached to pole. (B) View of the west plot on the second spray day showing the sand fly cages and droplet capture ribbons in position, with the Terminator ULV being operated from a truck bed; looking north west.

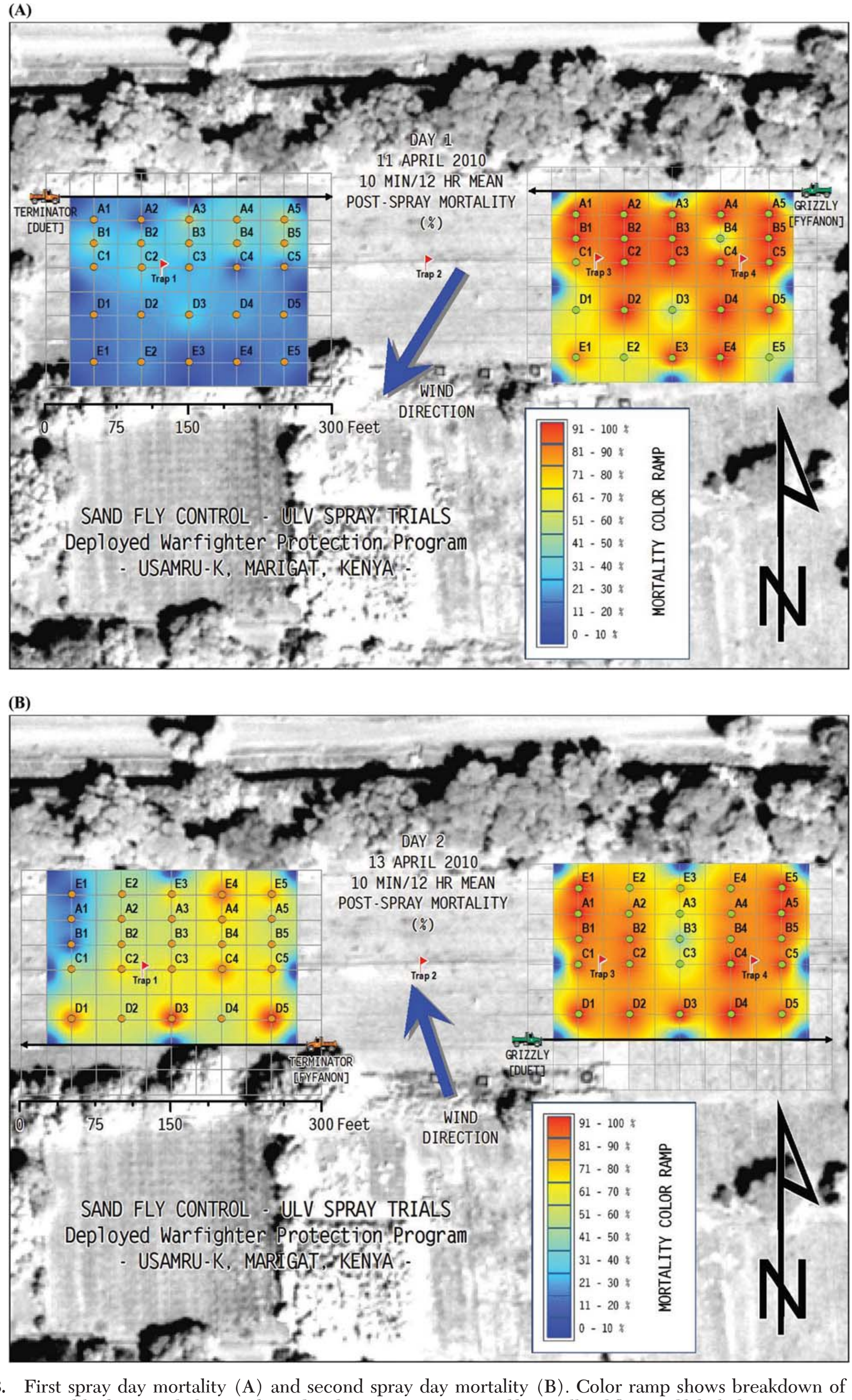


Fig. 3. First spray day mortality (A) and second spray day mortality (B). Color ramp shows breakdown of mortality frequency in 10% blocks. CDC light traps baited with CO_2 are represented by small red flags and labeled Traps 1–4. Two control CDC traps were located in a line to the east of Trap 4 on the other side of the tree line visible at the right edge of the map (see Fig. 1A).

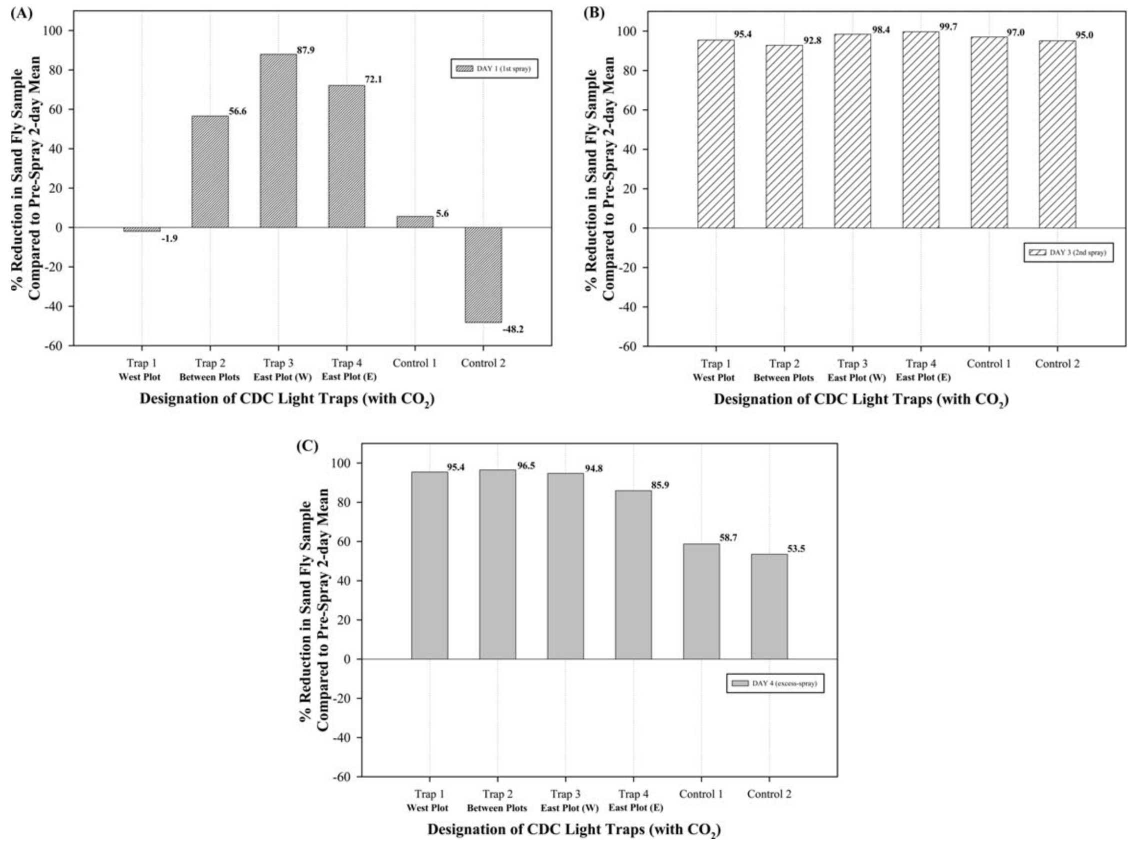


Fig. 4. Mortality observed in wild sand fly populations overnight immediately after the first spray day (day 1; A), overnight immediately after the second spray day (day 3; B), and overnight immediately after the excess Fyfanon spray day (day 4; C), based on CDC light trap data. Positive values represent reduction in sand fly numbers over prespray populations. Negative values represent an increase in sand fly numbers after spray trials.

west spray grid (Fig. 2B), and the Grizzly was run with Duet at 1.23 oz/acre from west to east on the south edge of the east spray grid. A change in wind direction, from the south-southeast on the second spray day, had forced us to flip each plot and driving direction to permit a simultaneous spray without cross-contamination (Figs. 1C and 3B). In addition, we had to move the E1 to E5 line of sentinel cages to a line north of the A1 to A5 line of sentinel cages to make room for the vehicles at the south edge of the spray grids.

Sampling Spray Droplet Deposition in the Test Grids. At 1 m from each sentinel cage position we inserted a second post for stretching a 1 m length of cotton ribbon parallel to the spray truck driving direction (Figs. 1 and 2). These ribbons were positioned to capture dye-labeled spray droplets at the same height as the sentinel cage at every sentinel cage position (Fig. 2A). This was an improvement from previous experiments (Britch et al. 2010a) where ribbons were only deployed at the central column of five cages perpendicular to the spray line. The unique alphanumeric code from A1 to E5 in each plot was copied on to each droplet sampling ribbon, along with the date and type of application. We later fluorometrically analyzed ribbons for deposition as described in Britch et al. (2010a).

Mapping Bioassay Efficacy Data and Inferred Active Ingredient Deposition. We mapped grids using 0.6 m resolution grayscale digital orthophotoquadrangles (GeoEye, Dulles, VA) in the ArcGIS 10 geographic information system (GIS; Environmental Systems Research Institute, Redlands, CA). We mapped bioassay mortality data on the image of the study area in the GIS by assigning the values of the mean of the 10 min and 12 h mortality frequencies to their respective pole positions and carrying out an inverse distance-weighted (IDW) interpolation analysis for each grid. Interpolation with IDW estimates a continuous surface using observed values from a defined set of points, and gradually constrains the influence of more distant points when estimating values at any given point. The IDW parameters were set to the default exponent of distance of 2, which controls the significance of surrounding points on an interpolated value, and an arbitrary fixed search radius of 400 feet from each interpolated point to take into account all values in a grid. To enhance visualization of the interpolated surface, we added null values to corners and midpoints of a square 7.6 m from the outermost observed values in the grid. We color-coded the resulting interpolated surface using a red to blue color ramp, where reds symbolized 80–100% mortality, yellows symbolized

60–80% mortality, greens symbolized 30–60% mortality, and blues symbolized 0–30% mortality (Figs. 3A and B). Similarly, we mapped inferred active ingredient deposition data on the grids using the fluorometric analyses of the droplet capture ribbons and used the same red to blue color ramp to partition inferred active ingredient deposition values on a scale of 0–1 $\mu g/cm^2$ (Figs. 5A and B).

Results

Weather Conditions. Winds were measured at 1–2 mph on both days, and the temperature on the first spray day was 31.1°C with 63.4% relative humidity (RH) at the time of spray, which was just after dusk at 1843 hours. The temperature and RH were not recorded on the second spray day. The second spray took place two days after the first spray day because of the absence of sufficient wind velocity on the day in between the two application days. The second spray was carried out at 1651 hours when winds were favorable, not waiting until dusk in case winds ceased as they had the previous day. At the start of the trial on the first spray day, wind speed from the north-east direction indicated by the blue line (Fig. 3A) was light and the direction fluctuated from north to north-east as the vehicles with ULV generators drove west to east for the Terminator and east to west for the Grizzly. At the start of the trial on the second spray day, wind speed from the south-east direction indicated by the blue line (Fig. 3B) was light and the direction fluctuated from south-west to south-east as the vehicles with ULV generators drove east to west for the Terminator and west to east for the Grizzly.

Patterns of Mortality in Test Grids. We found that sand flies from control sentinel cages were dying at 12 h postspray and in greater numbers at 24 h postspray (Table 1), most likely because of physiological stresses of living in the cages. Although control cage mortality was high at 12 h postspray in both trials, we observed several indicators that pesticide and not environmental exposure was responsible for the majority of mortality observed in cages from the spray treatment grids. First, mean 10 min postspray control cage mortality was 0% for the first spray compared with 80% in the Grizzly/Fyfanon plot, and mean 10 min postspray control mortality was 6% for the second spray compared with 33% in the Terminator/Fyfanon plot and 81% in the Grizzly/Duet plot (Table 1). These high values in most spray plots indicate rapid knockdown from the pesticide that was not observed in the controls.

Second, although the Terminator/Duet plot in the first spray showed 4% mean mortality at 10 min post spray (Table 1), barely higher than control mortality of 0%, many sand flies observed to be still moving but most likely intoxicated and moribund at 10 min in the Terminator/Duet plot, and thus not counted as dead, were among the dead at 12 h postspray in the Terminator/Duet plot. Moribund sand flies were not observed in control cages at 10 min post spray in either spray trial, but moribund individuals were observed in the Grizzly/Fyfanon, Terminator/Fyfanon, and Grizzly/Duet plots in both the first and second spray trials, which similarly contributed to mortality counts at 12 h.

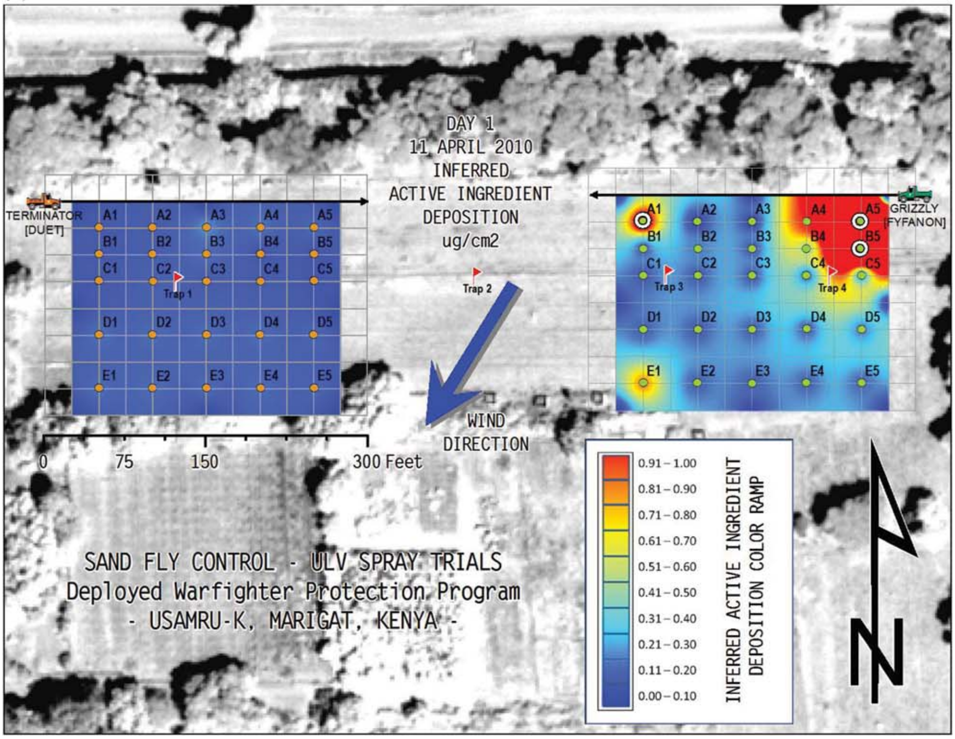
Third, 12 h mortality in control cages in both spray trials showed median values of 45% and 35% (Table 1); whereas, median values at 12 h post spray in treated cages were 100% mortality in three out of four cases. The fourth case was the Terminator/Duet application in the first spray trial that showed a median value of 40%. Although this value closely matched control cage median value of 45% at 12 h postspray, the IQR for the Terminator/Duet application was 45, indicating a wide spread of high and low mortality, compared with an IQR of only five in the 12 h post spray mortality for the control cages from the first spray trial. The wide spread in mortality values in the Terminator/Duet application was consistent with spatial variation in mortality across the grid consistent with an uneven application of pesticide (Fig. 3A); versus a narrow spread in mortality in control cages somewhat equally affected by environmental exposure.

Fourth, mortality values in treated cages at 12 h postspray generally matched mortality values at 24 h postspray, largely because most sand flies in most treated cages were dead at 12 h (Table 1). The one exception was the Terminator/Duet application in the first spray trial that, owing to the relatively low mean mortality of 48% at 12 h, showed nearly double the mean mortality at 24 h postspray, and a median value of 95% at 24 h. In contrast, mean control cage mortality between the 12 h and 24 h counts only increased by about half, with median values of 60% in both spray trials at 24 h.

To compensate for environmentally linked mortality in control cages that no doubt contributed to mortality in treated cages, we calculated the mean 10 min and 12 h mortality. As shown in Table 1 this function on the data provided a conservative perspective on efficacy of the machine/pesticide combinations. For instance, capturing the fact that applications with the Terminator had less initial knockdown than applications with the Grizzly, but tempering the apparent 100% 12 h kill across most of the grids in both of the Grizzly trials and one of the Terminator trials. The 10 min/12 h mean also created a correction closely comparable to Abbott's correction, but allowing some margin for moribund individuals at 10 min that were clearly affected by the pesticide. These 10 min/12 h mean mortality values were used to develop the mortality interpolations across the spray fields and visualize the relative efficacy of the different spray events as shown in Figs. 3A and B.

On the first spray day, looking at the 10 min-12 h mean mortality, we observed mortality >95% at over two thirds of the positions (range, 50–100%, mean 89%; Table 1) with the Grizzly/Fyfanon application on the east plot, compared with mortality lower than 40% at two thirds of the positions (range, 8–50%, mean 26%; Table 1) with the Terminator/Duet application on the west plot (Fig. 3A). The Grizzly/Fyfanon application in the east plot produced high mortality out to 175 feet from the spray line, impacting nearly every cage position with very high mortality. Conversely, the Terminator/Duet application

(A)



(B)

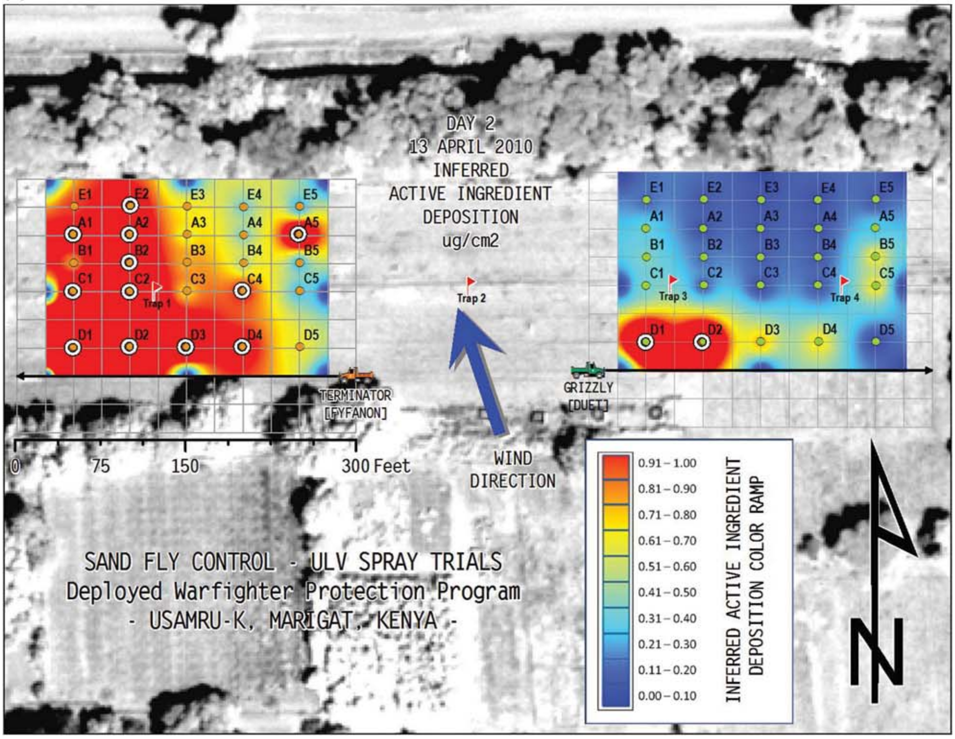


Fig. 5. First spray day (A) and second spray day (B) deposition of inferred active ingredient based upon fluorometric analyses of the fluorescent dye droplets captured on cotton ribbons adjacent to sand fly sentinel cages. Red color represents greatest deposition and blue represents lowest deposition. Units on color ramp are inferred $\mu g/cm^2$, and pole positions with inferred deposition values in excess of 1 $\mu g/cm^2$ are marked directly on the pole positions with a white ring.

Table 1. Mortality data from the two spray trials summarized by plot for mean, range, median, and interquartile range (IQR) using Microsoft Excel

Date	Time postspray/treatment group	Mean percent mortality (range, median, IQR)			10 min-12 h mean
		10 min	12 h	24 h	
11-Apr-10	W-plot Terminator/Duet	4 (0–25, 0, 5)	48 (15–100, 40, 45)	89 (45–100, 95, 20)	26 (8–50, 23, 23)
	E-plot Grizzly/Fyfanon	80 (0–100, 100, 30)	98 (60–100, 100, 0)	100 (100–100, 100, 0)	89 (50–100, 100, 15)
	Control	0 (0, 0, 0)	42 (20–60, 45, 5)	62 (45–80, 60, 15)	21 (10–30, 23, 3)
13-Apr-10	W-plot Terminator/Fyfanon	33 (0–100, 15, 45)	90 (10–100, 100, 0)	95 (35–100, 100, 0)	62 (8–100, 58, 23)
	E-plot Grizzly/Duet	81 (15–100, 90, 25)	97 (65–100, 100, 5)	98 (80–100, 100, 5)	89 (40–100, 93, 13)
	Control	6 (0–20, 0, 10)	40 (20–80, 35, 15)	64 (50–90, 60, 20)	23 (13–50, 18, 5)

The IQR, given in data units, is a measure of variance about the median, where low IQR values indicate a more consistent response to a treatment and high IQR values indicate a more variable response to a treatment.

in the west plot produced moderate mortality only out to 50 feet from the spray line, with two cages at the 25 feet line directly in front of the sprayer and the majority of cages throughout the grid beyond 50 feet showing very low mortality.

On the second spray day, looking at the 10 min-12 h mean mortality, we observed mortality >90% at nearly two thirds of the positions (range, 40–100%, mean 89%; Table 1) with the Grizzly/Duet application on the east plot, compared with mortality lower than 60% at more than half of the positions (range, 8–100%, mean 62%; Table 1) with the Terminator/Fyfanon application on the west plot (Fig. 3B). The Grizzly/Duet application in the east plot again produced very high mortality out to 150 feet from the spray line and impacted nearly every cage position with high mortality. The Terminator/Fyfanon application in the west plot also produced high mortality out to the 150 feet line, but the highest mortality was mostly constrained to the area south of a diagonal line from the north east to the south west corners of the grid, and, though every cage position was impacted, cages in the north west section of the grid showed very low mortality.

Patterns of Mortality in Local Populations. Natural populations of wild sand flies including *P. duboscqi*, *P. martini*, and *S. schwetzi* were significantly impacted by the experimental spray applications as measured by light traps baited with CO_2 placed overnight at the study site and in adjacent untreated areas (Figs. 4A–C). The data in Fig. 4 are shown as percentage reduction at six locations, Trap 1 to Trap 4 and Controls 1 and 2 (Fig. 1A), compared with the mean of two nights of pretreatment sampling at the same six locations. Positive histograms indicate population reductions; negative histograms indicate population increases.

Fig. 4A shows a near 2% increase in wild sand fly numbers at Trap one in the west spray plot (Terminator/Duet), over 50% reduction between the spray plots (Trap 2), 72–88% reduction in Traps 3 and 4 in the east plot (Grizzly/Fyfanon), and only about a 6% reduction in the control trap nearest the east plot (Control 1) the night after the first spray. The negative-48% change at Control 2 located farther to the east of the spray plots reflects a substantial population increase at that trap compared with the previous two nights before the spray (Fig. 4A). Figure 4B shows 93–100% reductions at all sample locations on the night immediately after the second spray, which was day 3 after the first spray. The next day, which was day 4 after the first spray, unused excess Fyfanon was applied at the label rate using the Grizzly in an 800 feet line along the north side of the study area with three mph winds from the north changing to northeast and temperatures 28.3–30.6°C. Populations sampled overnight after this spray (Fig. 4C) show continued 95–97% suppression in the west plot (Trap 1), the area between the plots (Trap 2), and the west-most trap of the east plot (Trap 3). However, the trap results in Fig. 4C also show a slight reduction in control down to 86% in Trap four in the east plot, and a distinct rebound to only 54–59% below prespray levels in the populations sampled by the two offsite traps, Controls 1 and 2, to the east in untreated areas (Fig. 1A).

Comparative Inferred Active Ingredient Deposition from ULV Sprayers. The interpolated inferred active ingredient deposition values are shown in Figs. 5A and B and raw inferred active ingredient deposition values in $\mu g/cm^2$ for the four spray runs are shown in Figs. 6A–D. The upper limit of deposition is not absolute, unlike mortality in the fixed population in a cage (i.e., 0–100%); however, 83 of the 100 inferred active ingredient deposition values were <1 $\mu g/cm^2$ and only two outlier values (10.34 and 12.46 $\mu g/cm^2$) were higher than 2.72 $\mu g/cm^2$. Therefore, for the purposes of visualization we capped the color ramp values at 1 $\mu g/cm^2$, and the partition of the color ramp in tenths is shown in Figs. 5A and B. All positions with values >1 $\mu g/cm^2$ are indicated on Figs. 5A and B with a white ring around the pole position point symbol. The two ribbon positions with the highest inferred active ingredient deposition values are position A5 in the east plot on the first spray day (10.34 $\mu g/cm^2$) and position D1 in the west plot on the second spray day (12.46 $\mu g/cm^2$). Both outliers resulted from Fyfanon, one from each ULV generator. The remaining 15 inferred active ingredient deposition values from 1.03 to 2.72 $\mu g/cm^2$ are also marked on the figures; the majority is found on the west plot on the second spray day from the Terminator/Duet application (see also Figs. 6A–D). Later analysis of the four ribbons soaked on-site in 20 ml of each of the four dye/pesticide solutions revealed that 12.12 g of dye added to 2 liters of Duet in the Terminator resulted in 1982 ppm of dissolved dye, 12.0 g added to 2 liters of Fyfanon in the Grizzly resulted in 3307 ppm, 8.0 g

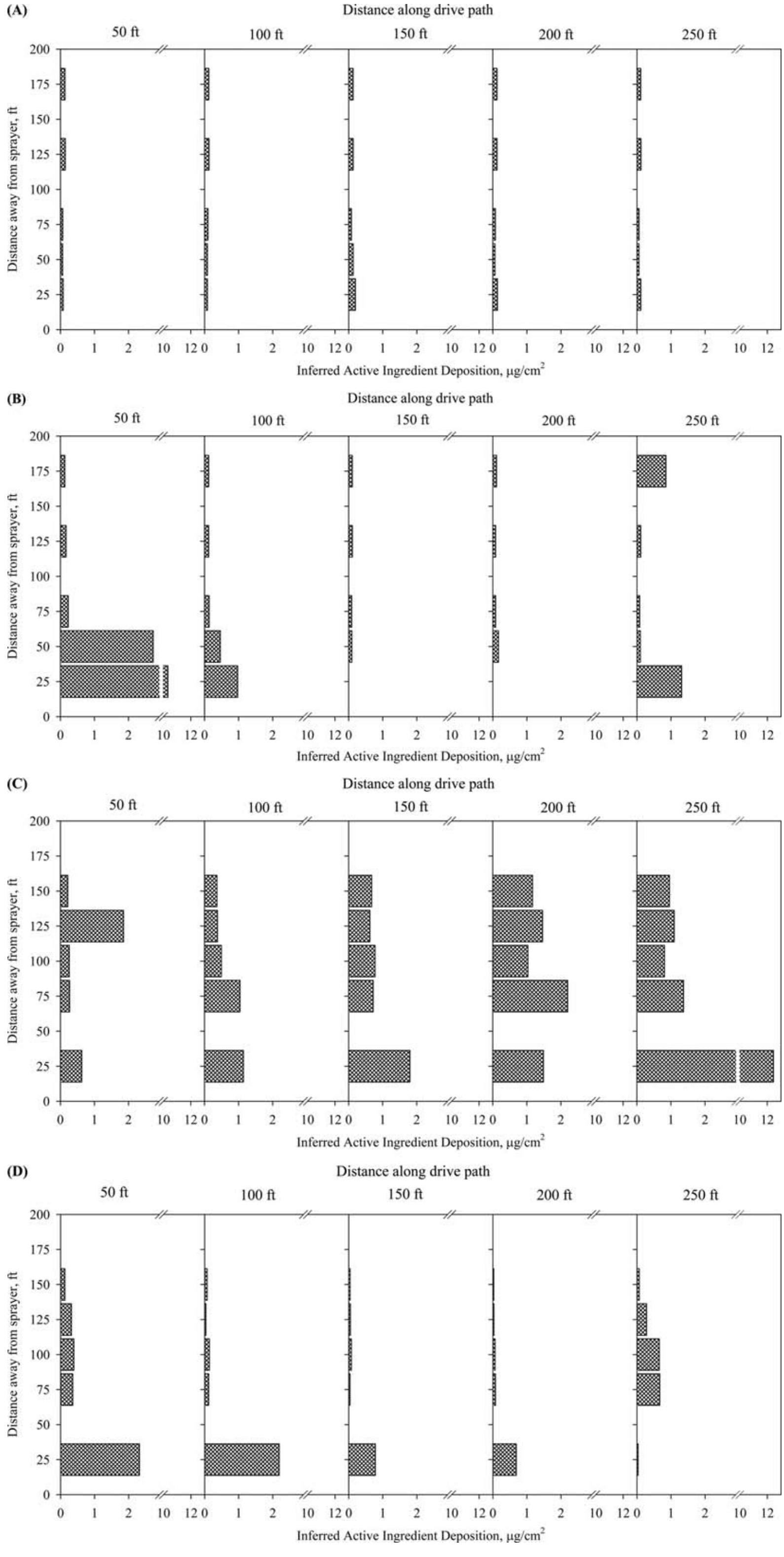


Fig. 6. Terminator/Duet Deposition of inferred active ingredient based upon fluorometric analyses at five distances from the 200 foot spray line for the first spray day Terminator/Duet (A), the first spray day Grizzly/Fyfanon (B), the second spray day Terminator/Fyfanon (C), and the second spray day Grizzly/Duet (D).

added to 2 liters of Fyfanon in the Terminator resulted in 9436 ppm, and 7.8 g added to 2 liters of Duet in the Grizzly resulted in 2416 ppm of dissolved dye. These values were used to calculate deposition for each spray grid as described in Britch et al. 2010a.

Reflecting the mortality effects from the Terminator/Duet run in the west plot on day one (Fig. 3A), the inferred active ingredient deposition values at that plot are uniformly near zero (Fig. 5A). However, unlike the very high mortality seen throughout the east plot on day one from the Grizzly/Fyfanon spray (Fig. 3A), the inferred active ingredient deposition values at that plot are mostly near zero but with a few maximum values near the spray initiation position in the north east corner of the grid, and a single maximum value near the spray termination position in the north west corner of the grid (Fig. 5A). Similarly, the inferred active ingredient deposition for the Grizzly/Duet spray on the second spray day was greatly focused on the first two ribbon positions near the spray initiation point and near zero throughout most of the remainder of the grid (Fig. 5B), not at all reflecting the uniformly high mortality observed throughout the grid (Fig. 3B). Conversely, the Terminator/Fyfanon run on the second spray day resulted in the most widespread inferred active ingredient deposition observed in the experiment (Fig. 5B), and though relatively low compared with Grizzly/Fyfanon- or Grizzly/Duet-induced mortality, the mortality in the Terminator/Fyfanon grid was observed nearly throughout the grid (Fig. 3B). However, the inferred active ingredient deposition maximum values in the Terminator/Fyfanon run were focused in the western half of the grid (Fig. 5B), whereas the mortality was focused more in the eastern half of the grid (Fig. 3B). Not surprisingly, a regression of inferred active ingredient deposition values with mortality values yielded no significant relationships in any of the four spray runs.

Discussion

This is the first known study looking at efficacy of ULV pesticide application on sand fly mortality in a natural setting. Ultra-low volume trials of Fyfanon (malathion) and Duet (prallethrin, Sumithrin, and PBO) on natural and caged populations of sand flies demonstrated substantial mortality. This study also demonstrated that the Grizzly ULV generator provided better overall mortality in the large field trial; however, the Terminator, which is much lighter and easier to handle, was also effective in producing sand fly mortality with Fyfanon. Fyfanon in both pieces of equipment and Duet, designed to incite activity to enhance efficacy of kill, produced mortality in both caged colony-reared populations as well as natural populations of Old World sand flies under hot dry conditions when applied by the Grizzly. Patterns of mortality throughout 150 feet by 200 feet or 175 feet by 200 feet grids of sentinel sand flies showed greater efficacy from the Grizzly ULV equipment regardless of chemical (Figs. 3A and B). However, patterns of deposition of active ingredient inferred from dye-labeled ULV droplets on cotton ribbons suspended adjacent to sentinel cages suggested a much greater dispersal of active ingredient in one trial from the Terminator ULV equipment (Figs. 5A and B) when applied with a very high concentration of dye (9436 ppm). Offsite sand fly trapping before and after the pesticide treatments suggests that local populations were suppressed from one or both of these ULV sprays (Figs. 4A–C).

The high and widespread inferred malathion deposition by the Terminator on the second spray day could have been because of the very long duration drive time or the high concentration of dye used (9436 ppm). The longer drive time was necessary because of the failure of one of the two ULV nozzles on the Terminator which required doubling of spray duration to apply the malathion at the label rate, and this would have allowed the environment more of an opportunity to disperse droplets. The questions remain, however, as to why the more widespread inferred active ingredient deposition by the Terminator on the second day did not result in a mortality pattern that matched the deposition, and why there was not a higher overall mortality given the high deposition. One possibility is that the active ingredient is separating from the dye in the spray, perhaps because of differential evaporation of the pesticide and the oil carrier in which the dye is dissolved, such that what we see, and what the ribbons capture, as a cloud of spray mist is not actually where the active ingredient is.

The reduction in volume of ULV droplets because of evaporation was recognized early in the development of ULV technology (Matthews 1977). Despite the emergence of oils as carriers to reduce evaporation (Wodageneh and Matthews 1981), oils will still evaporate (Woodrow et al. 1986), particularly in the hot-arid conditions in the current study. As droplets move through the air this evaporation will have the effect of creating smaller droplets with higher and higher concentrations of dye. Simultaneously, the active ingredient pesticides in the spray formulation may evaporate from these droplets. In this way, active ingredient may have a different spatial pattern of dispersal and concentration than may be inferred from the location and concentration of dye-labeled droplets. One possibility is that the active ingredient separates from the oil, or becomes concentrated in small amounts of oil, to the extent that a vapor of active ingredient is generated (Matthews 1977). This toxic vapor may disperse to more or different areas than the majority of oil droplets, and certainly to more or different areas than the dye-labeled droplets as may be seen by comparing Figs. 3 and 5. Wang (1991) found that only 16.8% of the application rate of malathion from a truck mounted ULV spray directed over a water impoundment was detectable in water samples at 24 min after the spray and suggested that the remaining malathion could have volatilized or otherwise degraded during the spray.

Another possibility is some gradient of immiscibility in the tank where the dye partially displaces active ingredient in the solution, settles toward the lower half

of the tank near the uptake, and is sprayed out before solution containing more active ingredient. Also, the Terminator has an enclosed tank from which the pesticide is drawn with Venturi suction to the spray nozzles, whereas the Grizzly draws from an open chemical reservoir with a pump. If the dye material mixed more efficiently or evenly in the enclosed Terminator tank, it could have led to the widespread dispersal of dye across the spray field seen on the second spray day. However, the dye in the Grizzly tank may have been more concentrated on the bottom of the pail and been blown out first and was gone, leaving a misleading picture of deposition on the ribbons when compared with mortality. In conjunction with this second scenario is that, looking at the wind directions on Figs. 5A and B, droplets containing more dye than active ingredient could behave differently in wind currents and thus move across the grids in a different pattern than droplets containing more active ingredient than dye. In addition, looking at the concentrations of the dye in the formulations in ppm compared with the amount of dye added in grams, it appears that the dye mixes more readily with the Fyfanon (see Results). This higher solubility may have led to higher overall deposition of dye when mixed with Fyfanon, and thus a biased estimate of active ingredient deposition, when compared with Duet regardless of sprayer (Fig. 5). In any case, the lack of congruence between inferred active ingredient deposition and mortality compels us to look more closely at how informative dye deposition measurements are in future experiments. In future experiments we should examine the possibility of eluting active ingredient from the cotton ribbons.

The CDC light trap data from the study area demonstrate that ULV applications are effective against local wild populations of *Phlebotomus* sand flies (Fig. 4). The suppression of populations sampled by the two offsite traps, Control 1 and 2 (Fig. 1A), overnight immediately after the second spray shows that spatial application of Duet in one area may have affected sand fly populations in adjacent areas (Fig. 4B). Yet, the lack of suppression in the west plot on the night immediately after the first spray points to a focal effect of the Fyfanon applied at the east plot, given that the Terminator/Duet application on the west plot produced little mortality. However, note that populations sampled in the offsite traps to the east, Control 1 and 2, immediately after the first spray were actually up to 48% *higher* than prespray levels (Fig. 4A), which could have been because of a repellent effect of one or both of the ULV sprays and a movement of sand flies to the east, perhaps enhanced by the winds that night from the north and north-northeast. Prallethrin, a major ingredient of Duet, has indeed been shown to have a repellent effect on sand flies (Sirak-Wizeman et al. 2008), and recent work demonstrates that 1% prallethrin on its own as well as in the Duet formulation bring about significantly increased movement behavior in *Lutzomyia shannoni* Dyar sand flies (S.A.A. and G.G.C., unpublished data). However, it did not appear that possible movement of Fyfanon from the east plot to the west by the wind affected populations sampled by the trap in the west plot (Trap 1) overnight after the first spray.

No light trap sampling was carried out on the night in between the two spray days, but on the night immediately after the spray of the excess Fyfanon, gradual increases in population samples in the two offsite traps to the east, Controls 1 and 2, point to a partial recolonization despite the application. This is not unlike the situation observed in the trap samples the night after the first spray day: after Fyfanon was applied in the east plot, sand fly populations appeared to increase in the offsite traps further to the east. A repellent effect of malathion on sand flies has not been reported. Although Achee et al. (2009) did not find a repellent effect of malathion with *Aedes aegypti* L., one study documented a repellent effect of malathion on oviposition behavior in *Ae. aegypti* (Moore 1977) and another study documented irritability in *Culex pipiens* L. by malathion, with irritability increasing in strains with higher resistance to the toxicant (Gaaboub and Dawood 1973). The lack of long-term control in Iraq despite repeated applications observed by Coleman et al. (2006) could have been because of recolonization from sand flies that had been repelled or driven from the areas sprayed with ULV pesticide applications, although migration from outlying areas or local population recruitment from larvae unaffected by the aerosol moving to adult stages in treated areas are possibilities also. A question arises as to whether an application of Duet on the third day would have kept the populations suppressed in the offsite areas. Certainly, the Duet application in the east plot on the second spray day appeared to have caused a much greater population reduction in the offsite areas to the east (Fig. 4B) than the Fyfanon in the east plot on the first spray day (Fig. 4A). However, under the scenario of Fyfanon causing a repellent effect, the population crash in the offsite traps, Controls 1 and 2, on the second night could point to sand flies that had moved away from the Fyfanon that were nevertheless mortally intoxicated at low level with a delayed effect.

The dual action nature of Duet may be very important in sand fly control at this study site. Note that the second set of sprays on 13 April were carried out over 1.5 h earlier in the day than the first set of sprays on 11 April, well before what was thought of as the beginning of activity for sand flies in this region (i.e., 1800 hours, based on timing of experiments conducted in Marigat by Mutinga et al. 1986 and Mutero et al. 1991). If it is true that the majority of sand flies were at rest at the time of the second set of sprays, then the Duet formulation was reaching sand flies in their resting areas, inducing them to fly and have more and more droplets impinge on them. In earlier work, ULV spray clouds have been observed to spread faster and to larger areas just before inversion starts, i.e., ≈1 h before sunset (M. F., unpublished data). If it is true that the two offsite traps were measuring population suppression because of Duet, then the Duet was moving well outside of the area of application, with wind currents, thus spatially multiplying the effective reach

of the application. Basimike and Mutinga (1992) described sand fly habitat in this area to principally include rodent burrows and termite mounds. Rodent burrows and at least two large termite mounds were observed in the study site (Fig. 2A, inset). Termite mounds are constructed by the termites to exploit passive convection of air currents and this air flow may have drawn enough Duet into the mounds to cause sand flies to exit the mounds, or at least move around in them, and become sufficiently intoxicated to reduce populations available to move to light traps later that night. However, the majority of the Duet must have dispersed through the surrounding vegetation, indicating that sand flies are probably resting in many more places than subterranean larval habitat areas.

An issue that should be discussed with regard to choosing ULV generators for military spray missions is the susceptibility of the performance of the equipment to weather. The Grizzly produced a spray cloud with inherent velocity; for example, the spray blast reached directly to the second row of cages 50 feet from the spray line during calibration tests with little or no wind present. The Terminator did not exhibit a blasting effect when producing its ULV spray cloud, and the spray cloud movement was thus more dependent on the environment. The higher air blast created by the Grizzly also helps in better mixing of the spray into the air space across the grid. These phenomena are illustrated in Figs. 3A and B where mortality from Grizzly applications penetrates the farthest points of the sentinel grid, unlike the mortality from Terminator applications that do not. Based on the mortality patterns, the heavier, gasoline powered Grizzly produced a more consistent, widespread, and effective kill when compared with the lighter, diesel powered Terminator.

However, for military contingency operations in austere hot-dry environments, the Terminator is still a viable choice: two people can move and carry this compact device, which is an extremely important factor when transporting pest control equipment to remote locations on helicopters and armored vehicle convoys; diesel fuel will be available in more remote locations than gasoline; remote outposts frequently have ATVs on site; and many remote military outposts, where risks of exposure to sand fly *Leishmania* vectors may be highest, are small enough that the Terminator could be effective at providing area control for limited periods of time. For military operations in larger garrison-like hot-dry locations, the Grizzly would be the best choice of the two ULV generators tested here: larger contingency bases have gasoline fuel points, motor pools with pickup-truck sized vehicles on which the unit could be permanently affixed, and very large areas that need to be treated to provide effective relief from pest and disease vector insects. As discussed in Britch et al. (2010a), one concern that should be noted is that in combat zone pest management scenarios there may only be one opportunity to conduct a pesticide mission because of constraints of transportation, personnel, scheduling, enemy activity, or severe weather, and despite the inconvenience of a larger ULV generator it may be a wiser choice.

There are important applications of these positive findings for future military preventive medicine activities and we encourage further investigations into the merits of ULV for enhancing force health protection against sand flies. Current DoD doctrine emphasizes the use of permethrin treatment of uniforms and use of DEET on exposed skin as the primary defense against sand fly bites, but studies show that permethrin treatment of uniforms on its own may not be an effective way to prevent or even reduce cases of cutaneous leishmaniasis in soldiers (for example, Asilian et al. 2003). Any control program against sand fly vectors of *Leishmania* should consist of a suite of control factors. For instance, Mutinga et al. (1992) showed that permethrin-impregnated wall cloth could reduce endophilic sand fly species in Kenyan rural homes by as much as 85%. Combining ULV treatments of natural sand fly populations, shown here to be effective, with control factors like treated wall cloths, treated vegetation (Britch et al. 2009), treated camouflage netting (Britch et al. 2010b), permethrin-treated clothing, and use of DEET, could substantially minimize opportunities for human-sand fly contact and thus minimize opportunities for transmission of *Leishmania* to deployed troops.

Acknowledgments

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